REMARKS

Claims 1-20 are pending in the present application.

Applicants wish to thank Examiner Sakelaris and Examiner Fredman for the helpful and courteous discussion with their undersigned Representative on May 29, 2003. The content of this discussion, as well as the issues raised therein, are summarized and expanding upon more fully below.

The rejection of Claims 1-3 under 35 U.S.C. §103(a) over Kinzler et al in view of Okubo et al is obviated in part by amendment and traversed in part.

In the rapidly exploding world of genomic research, a wealth of putative genes and proteins are being identified and indexed. Although the indexes (databases) are extremely helpful for determining the identity and/or activity of a putative gene and/or protein, these databases provide little if any direction with respect to the expression patterns and expression frequencies of the genes. To further confuse the issue, not all genes in a cell are transcribed into mRNA and ultimately translated into proteins. It is estimated that the human cell will express approximately 15,000 genes with the expression frequency of each gene responding to several environmental factors, such as cell type and external stimuli.

Current efforts have been focused on measuring the total types of mRNA; however, measurement of genetic expression frequency information based on total proteins has remained elusive. Heretofore, the most effective means for genetic expression profile analysis has been the method of serial analysis of gene expression (a.k.a. SAGE) as set forth by Kinzler et al (U.S. 5,695,937).

As set forth in Kinzler et al (see columns 3-8) and summarized in the present specification, in SAGE cDNA is produced by using a poly(T) having the 3' end bonded with biotin as a primer, the cDNA is digested with a restriction enzyme (a.k.a. an "anchoring enzyme"), the cDNA fragments containing the 3'-biotinare adsorbed on avidin beads, the beads are divided into two portions, and two distinct linkers (A or B) are ligated to the cDNA fragments (about 13 bp) adsorbed on either portion of the divided bead population (see page 5, line 27 to page 6, line 8 of the present specification). Each linker (A or B) contains a site for a Class II restriction enzyme, termed a "tagging enzyme" (see page 6, lines 8-10 of the present specification). The cDNA fragments are then excised from the beads with the tagging enzyme, blunt-ended at the excised end, and the tags are ligated to the linker A and the liner B are connected (see page 6, lines 10-14 of the present specification). Accordingly, the product of this connection is termed a "ditag" (see page 6, lines 14-15 of the present specification). Subsequently, the ditag is amplified by PCR using primers that recognize linker A and linker B and a large number of amplified ditags are ligated, inserted into a vector, and sequenced (see page 6, lines 15-19 of the present specification). Accordingly in the SAGE method, 50 tag sequences can be determined by one round of sequencing from which the expression frequency may be calculated (see page 6, lines 19-22 of the present specification).

However, the SAGE method disclosed by Kinzler et al is saddled with many problems and/or deficiencies, as the method could not be reproduced in most research facilities (see page 7, lines 10-13 of the present specification). For example, the SAGE method is very complicated and requires specially trained people to administer. Moreover, for each measurement nearly 1 μg of mRNA is required, which is nearly impossible to obtain from clinical biopsies or micro tissue samples (see page 7, lines 16-22 of the present specification).

Further, the SAGE method is prone to errors due to its reliance on the accurate determination of the sequence of a very short (13 bp) tag (see page 7, lines 23 to page 8, line 5 of the present specification). A significant source of the errors affecting the applicability of the SAGE method arises from the fact that the SAGE method requires the formation of a ditag resulting in a poorly defined border region. Specifically, due to the nature of the restriction enzymes selected, the tags that are interconnected to form the ditags inherently are of different lengths and become intermingled. Therefore, upon ligation it is extremely difficult to determine where one tag ends and the next one begins.

Another source of errors associated with the SAGE method is the use of an avidin/biotin capture method. The avidin/biotin capture method lends to an increase in contamination further increasing the requirement for an increased quantity of mRNA to obtain data.

As stated in the present specification at page 9, line 12 to page 10, line 2, in actual laboratory practice, the SAGE method possesses many drawbacks, which includes:

1) the techniques required for the method are complicated and they can be performed only by specially trained persons;
2) about 1 μg of mRNA is required for the measurement, and therefore it is substantially impossible to perform the measurement with a sample that can be obtained in a small amount, for example, a clinical biopsy material, and it is similarly impossible to measure difference of genetic expression in micro tissue portions; and
3) the method theoretically causes considerable measurement errors because a ditag is measured.

Okubo et al merely provide an arrangement of restriction sites with respect to the 3' poly(T) sequence, therefore Okubo et al does not compensate for the myriad of deficiencies in the disclosure of Kinzler et al

In order to solve these problems associate with the art of record, Applicants have surprisingly developed the following method for analyzing expression frequencies of genes called MAGE (Micro-Analysis of Gene Expression):

(a) a step of forming a vector primer to which each cDNA is ligated, by annealing the vector primer with each mRNA derived from a cell of which expression frequencies of genes is to be analyzed, and synthesizing the cDNA, said vector primer comprising a linear plasmid vector having a single-stranded poly(T) sequence at one 3' end, a recognition sequence for a first restriction enzyme at an upstream position from the poly(T) sequence, a recognition sequence for a second restriction enzyme near the 5' end of the strand having the poly(T) sequence, and a recognition sequence for a type IIS restriction enzyme at an downstream position from the recognition sequence for the second restriction enzyme,

(b) a step of digesting the vector primer to which the cDNA is ligated, with the second restriction enzyme and a third restriction enzyme that does not digest the vector primer and forms a digested end of the same shape as a digested end obtained with the second restriction enzyme, to excise an upstream region of the cDNA, and cyclizing the vector primer,

(c) a step of digesting the cyclized vector primer with the first restriction enzyme and the type IIS restriction enzyme to excise a downstream region of the cDNA so that a tag consisting of a part of the cDNA is left, and cyclizing the vector primer again,

(d) a step of performing polymerase chain reaction (PCR) by using the vector primer as a template and primers to amplify the tag, wherein said primers are oligonucleotides having nucleotide sequences corresponding to known nucleotide regions on each side of the tag that are maintained in the vector primer following digestion in step (c),

(e) a step of ligating the amplification products to form a concatemer of the tags, wherein the tags are separated by known nucleotide sequences introduced by the primers for tag amplification so that ***no ditags are present in the concatemer***, and

(f) a step of determining the nucleotide sequence of the concatemer and investigating types and frequencies of tags occurring in the nucleotide sequence (see Claim 1).

Applicants wish to draw the Examiner's attention to the fact that the claimed invention (above) is neither disclosed, nor suggested by the combined disclosures of Kinzler et al and Okubo et al. Specifically, Kinzler et al requires the formation of ditags (see Claims 1-43 and column 2, line 21 to column 14, line 22), whereas the present invention has specifically excluded the formation of ditags (see Claim 1, step (e)). Such a disclosure by Kinzler et al *teaches away* from the claimed invention. Furthermore, Okubo et al merely provide an arrangement of restriction sites with respect to the 3' poly(T) sequence and does not compensate for the teaching away from the present invention by Kinzler et al.

Moreover, the present invention, in which a ditag is not produced, solves the prior art problems of the indefinite border between tags. Since a sample can be amplified by PCR several times, the present invention permits the analysis of sub-micro quantities of mRNA (see page 27, line 9-12 of the present specification). In addition, since the present invention uses a vector primer for cDNA synthesis, the cDNA may be directly fused to the vector and, therefore, analysis can be performed without using an avidin/biotin capture system, thus avoiding the introduction of additional contaminants that plagued the SAGE method (see page 27, lines 12-16 of the present specification).

Citing In re Royka, 490 F.2d 981, 180 USPQ 580 (CCPA 1974), MPEP §2143.03 states: "To establish a prima facie obviousness of a claimed invention, all the claim limitations must be taught or suggested by the prior art." Applicants submit that the

combined disclosures of Kinzler et al and Okubo et al fail to meet this requirement, and as such the artisan would have no direction to practice the claimed method in which ditags are not formed in the concatemer, much less the advantageous properties flowing therefrom. Accordingly, the present invention would not be obvious in view of the combined disclosures of Kinzler et al and Okubo et al.

Applicants respectfully request withdrawal of this ground of rejection.

The rejection of Claims 1-3 under 35 U.S.C. §112, second paragraph, is obviated by amendment.

Applicants have amended the claims to provide proper antecedent basis for all terms and to clarify the meaning of the objected to terms.

Withdrawal of this ground of rejection is requested.

The objection to Claims 4-8 under 37 C.F.R. §1.75(c) is obviated by amendment. Applicants have amended the claims to remove improper multiple dependencies.

Withdrawal of this ground of objection is requested.

The Examiner has indicated that the claim to foreign priority has not yet been granted because the foreign priority documents have not yet been received (see Office Action of March 25, 2003, page 1, paragraph 13 and page 2, lines 3-6). Applicants note that the present application is a 371 of PCT/JP00/00902 filed on February 17, 2000. As indicated on the enclosed copy of the Request for Priority under 35 U.S.C. 119 and the International Convention (filed on August 16, 2001), a certified copy of the corresponding Convention application, JP 11-038538 (filed on February 17, 1999), was submitted to the International

Bureau in PCT Application No. PCT/JP00/00902. Moreover, receipt of the certified copy by the International Bureau in a timely manner under PCT Rule 17.1(a) has been acknowledged as evidenced by the attached PCT/IB/304, also filed on August 16, 2001. Accordingly, Applicants note that the obligation to obtain the priority document from the International Bureau pursuant to PCT Rule 17.2(a) is upon the Office (see MPEP §1893.03(c)).

Applicants request that, in the next communication, the Office acknowledge their claim to priority and acknowledge that copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau.

Applicants submit that the present application is now in condition for allowance. Early notification of such action is earnestly solicited.

Respectfully submitted,

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